AN EVALUATION OF SOME METHODS FOR DETECTING LOW LEVELS OF INFECTION AND INOCULATION-RECOVERY RELATIONSHIPS OF *TRICHINELLA SPIRALIS* IN SWINE

by

MAX ELMER COATS JR.

D. V. M., Colorado State University, 1963

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Pathology

Department of Infectious Diseases

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1969

Approved by:

Stanley E. Leland Jr.

Major Professor

TABLE OF CONTENTS

Page

INTRODUCTION 1

REVIEW OF THE LITERATURE 4

MATERIALS AND METHODS 13

Source and Maintenance of Infection 13
Sex Determination of Larvae 13
Experimentally Infected Pigs 14
Infectivity Control 15
Tissue Examination of Experimentally Infected Pigs 15
Charcoal Card Agglutination Testing 17
Gel Diffusion Antigen Preparation 19
Culture of Trichinella spiralis 20
Gel Diffusion Testing 21

RESULTS 22

Sex Determination of Larvae 22
Infectivity Control 22
Tissue Examination of Experimentally Infected Pigs 23
Charcoal Card Agglutination Testing 24
Culture of Trichinella spiralis 25
Gel Diffusion Testing 25

DISCUSSION 26

CONCLUSIONS 30

ACKNOWLEDGMENTS 31

LITERATURE CITED 32

INTRODUCTION

Trichinosis is a parasitic disease which occurs in a wide variety of animals including man. The disease in man may be inapparent or severe enough to cause death in three to six weeks. In swine the disease is mild and improved methods of detection are desirable since inadequately cooked pork products are the main source of infection for man.

The causative agent Trichinella spiralis was discovered in human muscle tissue taken at autopsy by a young medical student, James Paget, in 1835. Since its discovery, this parasite has been recovered from the tissues of more than 75 species of land dwelling and aquatic animals including some avian species (Zimmermann and Hubbard, 1963). Feces of certain species of animals may also contain infective larvae (Zimmermann, 1966).

When a parasite is capable of living successfully in a large number of different host-species, the epidemiology of the disease caused by the parasite becomes complex. Man normally becomes infected by ingesting pork or pork products containing viable infective larvae.

Ideas concerning the best method of dealing with trichinosis in swine are varied and range from having all people abstain from eating pork to employing a method of microscopic inspection of samples from all slaughtered pigs. There are several methods which kill the parasite in products which may be eaten without additional cooking. Approved freezing, cooking, and drying and salting procedures are outlined in the Regulations Governing the Meat Inspection of the United States Department of Agriculture (Service and Regulatory Announcements C & MS-SRA-188).

Early in the history of organized meat inspection in the United States, the decision was made not to inspect all swine carcasses as some of the

European countries were doing. Currently, there is a renewal of interest in the eradication of trichinosis from swine in the U. S. Many reasons for the increased interest have been proposed. They range from the humanitarian's desire to reduce human disease and suffering to the businessman's endeavor to increase both domestic and foreign consumption of United States pork and pork products.

Eradication is an excellent goal but before any serious effort can be made, reliable tests for trichinosis of swine will be necessary. Tests must detect recent and long standing infections at high and low levels. In addition, methods must be available which can be used to detect infection both ante mortem and post mortem. In order for a test or tests to be the basis of a large scale program it would have to meet the following requirements:

1. be specific.
2. be sensitive at any level of infection.
3. be simple to perform with minimal training.
4. be reasonably rapid (less than 24 hours).
5. be relatively inexpensive.

Sensitivity of any test must be firmly established if the test is to have a place in either control or eradication of trichinosis. Data is needed to help establish the lower limits of sensitivity of the detection methods currently available. Data on inoculation-recovery (larvae/gram tissue) relationships would aid in determining the number of infective larvae swine ingest in naturally occurring cases of trichinosis. This information assists epidemiologists in determining the significance of various potential sources of infection.

This study examines the following:

1. Inoculation-recovery relationship of Trichinella in swine at low dose levels.
2. Sensitivity of the Charcoal Card Test (Anderson et al., 1963) in detecting very light infections in swine.
3. Culture of T. spiralis as a source of test antigens.
4. Sensitivity of a gel diffusion technique as a method of detecting swine with very light infections.

REVIEW OF THE LITERATURE

Descriptions by anatomists' writing in the early 1800's may have been the first record of trichinosis in man but credit for the discovery of Trichinella spiralis has been given to James Paget who detected the cysts in the muscle of a man who died of tuberculosis. His discovery was made in 1835. The name, Trichinella spiralis, was proposed by Sir Richard Owen. The discovery of trichinosis in animals was made by Joseph Leidy in 1845. He demonstrated the parasite in cooked pork.

Transmission by ingestion of infected flesh was demonstrated in 1850 by Herbst, a German worker, who infected a badger with trichinous dog meat. Zenker, a German physician, became involved with a small scale epidemic of trichinosis in 1860. During his investigation, he demonstrated the source of the infection (infected pork) and correctly postulated the mode of spread within the host's body.

McCoy (1938) reported experimental fecal transmission of Trichinella using rats. Spindler (1953) demonstrated fecal transmission of T. spiralis from dogs to pigs. Since that time, several other workers have also investigated this mode of transmission and have confirmed and enlarged upon the early reports. Intra and inter species transmission by contaminated feces has been demonstrated.

Transplacental transmission was reported by Denham (1966) who also found that infection of nursing mice by ingestion of milk from infected mothers occurred.

Experiments have been conducted in an effort to determine the size of the average infecting dose that produces naturally occurring cases of trichinosis in swine. The infecting dose for swine proposed by Hill (1957)

was 400-600 larvae/lb body weight. In 1966, however, Scholtens et al. after reviewing the subject suggested that commonly occurring levels of infection in swine might result from a total dose of as few as 10 larvae.

T. spiralis is unusual among nematode parasites because of the tremendous variety of animals which can be used successfully as hosts. Gould (1945) has compiled an extensive listing of both natural and experimental host species. The natural hosts range from walrus and white whale (Kagen, 1960) to mongoose and hippopotamus (Cram, 1941). Cram also reported on experimental infections in chickens, cattle, horses, pigeons and magpies.

Chandler (1960) reports that Trichinella is almost absent in the tropics. T. spiralis is found primarily in Europe, the United States and Arctic regions but is only moderately common in Mexico and South America. Some differences between geographical strains are reported (Kozar, 1965).

Most (1965) reviewed the history of human trichinosis in the U. S. and reported a definite decline in cases between 1940 and 1965. In a recent publication dealing with the status of human trichinosis (Annon., 1968) the 20 year period 1947-1967 was reviewed and the following figures reported:

1. In 1947 451 cases occurred with 14 deaths.
   In 1957 178 cases occurred with 4 deaths.
   In 1967 67 cases occurred with 0 deaths.
2. In 53 of 67 cases in 1967, pork products were incriminated as the source of infection and in 44 cases the source of the meat was determined. All 44 cases were due to meat from commercial sources.
3. Recent autopsy studies found 4.2% of diaphragms infected as compared with 16.1% found during the period 1931-1942.
4. The most recent report shows 1.6% incidence in persons under 44

years old compared with an incidence of 4.5% for those over 45 years of age.

Zimmermann and Brandly (1965) reported on the status of trichinosis in swine in the U. S. based on a survey conducted from 1961-1965. Using statistical methods, herds and individuals were selected. The incidence and severity (larvae/gm diaphragm) were determined. In all, 21,417 diaphragms were examined and the following rates of infection were calculated: butchers, 12/10,000; breeders, 22/10,000; garbage fed, 260/10,000. The authors stated that infection rates appeared to be dropping steadily during the survey period. The degree of infection was reported as follows:

LARVAE/GM OF DIAPHRAGM	1	1-10	11-50	51-500	Over 500
Butcher	100%	--	--	--	--
Breeder	92%	8%	--	--	--
Garbage fed	60%	23%	8%	7%	2%

Distribution of Severity of Naturally Occurring Trichinella Infection in Various Types of Swine.*

*(The original figures have been converted to percent.)

Jefferies et al., 1966 estimated the incidence of trichinosis in U. S. swine at 0.5% ± 0.25% (confidence interval 95%). The severity (larvae/gm diaphragm) was reported as follows:

LARVAE/GM OF DIAPHRAGM	1	1-10	Over 10
Percent of Positive Samples	60	24	16

Severity of Naturally Occurring Trichinella Infection in Swine.*

*(The original figures have been converted to percent.)

In the U. S., the incidence of trichinosis in wildlife has been studied extensively in only a few areas. Zimmermann and Hubbard (1963) reviewed wildlife trichinosis in Iowa and based on previous reports, they state that 7.3% of foxes, 6.4% of rats and 5% of mink checked during the period 1953-1963 were trichinous. Tissue digestion was the testing method used.

The importance of wildlife reservoirs is emphasized by a report (Podhajecky, 1964) on minimum infecting dose for mice which states:

1. Two larvae per dose resulted in no infections in an undisclosed number of mice.
2. Three larvae per dose resulted in 1 infection in an undisclosed number of exposed mice.
3. Five larvae per dose resulted in 5 infections in 10 exposed mice.
4. Six larvae per dose resulted in 10 infections in 10 exposed mice.

There is currently some disagreement between authorities on the matter of the life cycle of this parasite. Berntzen (1965) presents the most convincing evidence for his proposition. He states that the life cycle is typical of all nematodes. It consists of 4 larval stages and an adult stage. Adults develop within 24 hours after ingestion of infective material and mating begins as early as 48 hours after infection.

The eggs embryonate *in utero* and this first stage larvae molts *in ovo*, then immediately hatches. This second stage larvae is deposited in the mucosa and from there migrates to the muscle. The second stage larvae may be found in the muscle between 7 and 26 days after the host ingests cysts. These larvae molt to become third stage between 11 and 35 days post infection but do not migrate further. One more molt occurs and the resulting fourth stage larvae become encysted. The entire process may be completed

in 15 days. Larvae within the cysts remain alive for an undetermined time.

Early workers tried to cultivate Trichinella in vitro. McCoy (1936) tried to grow the organism using a Maitland type culture but was unable to get either growth or sexual differentiation. Some growth and organogenesis was reported by a worker using a roller tube tissue culture technique (Weller, 1943). Sexual differentiation with reduction in size has been reported (Kim, 1961, 1962). Reports have been published which state that it is possible to culture Trichinella spiralis from excysted larvae to adults (Berntzen, 1962, 1965).

One of the several reasons for interest in in vitro cultivation was the opportunity it would afford to isolate and study metabolic by-products and determine their possible role in acquired immunity. Detailed studies were conducted in an effort to determine the role of excretions and secretions of Trichinella larvae and adults in immunity (Campbell, 1955; Sadun and Norman, 1957). A study of the nature of the excretions and secretions was made by Mills and Kent (1965). These excretions and secretions were used as antigens in various serological procedures and a standard method of preparation was described (Annon., 1967).

Trichinosis in man may be inapparent or result in clinical illness. In the clinical form, symptoms are inconsistent. Edema of the eyelids is prominent. Gastrointestinal symptoms may occur. Muscle soreness, skin lesions, hyperhydrosis, chills, fever and eosinophilia may occur. The clinical form may be confused with acute food poisoning, cholera, influenza, typhoid or other forms of dysentary. The disease may be fatal (Gordon, 1965).

According to Strafuss and Zimmermann (1967), single doses of 5,000,

one hundred thousand and 400,000 larvae failed to produce clinical illness in swine. Rate of gain was slightly decreased in pigs receiving 400,000 larvae. All pigs were afebrile even though one had a 4 day period of diarrhea.

The disease is difficult to diagnose with certainty. The earliest method of arriving at a positive ante mortem diagnosis of trichinosis in man was by biopsy (Friedreich, 1862). Modifications of this method are still in use today. Muscle tissue may be examined by any of the following methods:

1. Compression of thin pieces of muscle tissue between glass slides and direct examination with a microscope fitted to a projector (Trichinoscope projection) is used in some European countries.
2. Microscopic examination of fixed, stained sections of muscle tissue can be made.
3. Digestion of the tissue using the acid-pepsin digestion technique first described in 1897 by Thornbury or a modification thereof and subsequent examination of the digest for larvae is a common method.
4. A masceration technique (Levine, 1941) was suggested if infection of less than 21 days duration was suspected.

Schwartz and McIntosh (1929) investigated skin testing methods in experimentally infected pigs, but despite continued efforts by many workers, Gould (1945) stated that skin testing in swine was not a reliable procedure.

The search for a satisfactory serological test began with compliment fixation (CF) proposed by Strobel in 1911. As new antigens were prepared, this test was modified but the CF test was never entirely satisfactory.

Bachman (1929) demonstrated a precipitin reaction but this test was reported to be unreliable when testing swine (Spindler et al., 1941). Oliver-Gonzales (1940) described a microprecipitin test which used living larvae. A flocculation test (SK) was described by Suessenguth and Kline in 1944. Many variations on this method were soon published. These modifications were reviewed by Kagen (1960).

Bozicevich (1951) introduced a test using bentonite coated with a water soluble antigen. This became known as the bentonite flocculation (BF) test. Hemagglutination (Kagen and Bargai, 1956) and slide hemagglutination (Sadun and Allain, 1957) were the next types suggested. Innella and Redner (1959) devised a test using latex particles which had been coated with antigen. This test became known as the latex agglutination (LA) test.

The florescent antibody (FA) test was proposed and evaluated by comparison with the CF and BF (Sadun et al., 1962). The FA test was reported to have a high degree of sensitivity and specificity when testing humans or rabbits. Comparisons of various commercially available test reagents were made (Norman, 1963). The test methods were LA, SK, and the BF. The authors concluded that the BF test was unsatisfactory for detection of either light infections or infections of long standing in humans. The LA was recommended for use when only occasional samples were tested. The SK test was said to be too sensitive for use on humans.

Anderson and his co-workers (1963) adapted the plasma reagin card test for syphilis (Portony, 1962) for use in the diagnosis of trichinosis. This new technique, the Charcoal Card Test (CCT), was used in evaluating sera from humans and rabbits. The antigen was an acid soluble fraction of T. spiralis larvae (Melcher, 1943), absorbed onto cholesterol-lecithin crystals

and then combined with a special charcoal suspension. The National Communicable Disease Center (N.C.D.C.) reported on an evaluation of several tests conducted during the investigation of an outbreak of trichinosis (Lamb et al., 1964). The report compared the results of testing using BF, LA and CCT. Of 71 samples tested, 43 were BF positive, 38 were LA positive and 39 were CCT positive.

A further evaluation of the SK test was made (Suessenguth et al., 1965). The SK test detected 51 of the 53 swine in the experiment. The time required to become positive was proportional to the dose (i.e. dose 100 larvae - positive at 50 days; dose 500 larvae - 37.7 days; dose 1,000 larvae - 32.2 days). The larvae per gram were reported as follows:

Dose 100 larvae - recovery 13/gm diaphragm;

Dose 500 larvae - recovery 38.2/gm diaphragm;

Dose 1,000 larvae - recovery 93.2/gm diaphragm.

A report evaluating the BF, LA, FA, SK, and CCT tests was prepared by workers at N.C.D.C. (Scholtens et al., 1966). The purpose of their work was to: (1) evaluate the efficiency of the various tests in their ability to detect several levels of infection in swine and (2) determine the larval dose required to produce infections yielding about 1 larvae/gm of diaphragm in swine.

Doses used in this experiment were 500 larvae, 1,000 larvae, and 100,000 larvae. Eight serum samples from each animal were tested between 7 and 90 days after infection. The time from infection until positive serological results were obtained varied, but all became positive between 21 and 28 days after infection. The time required before infected pigs reacted positively and the length of time infected pigs remained positive was comparable for

the FA and CCT.

The BF test was not as effective as the other methods in detecting trichinellosis in swine. Inoculation-recovery figures from this and other articles were analyzed by plotting the data on a graph. The projection of this line indicates that as few as 10 infective larvae may be responsible for the average infection in domestic swine. Sulzer and Chisholm (1966) also reported an evaluation of the BF, LA, SK, CCT and FA tests. Their report indicates that the CCT and FA tests were the most effective in detecting trichinosis in swine.

Over the years many methods of preparing Trichinella antigens have been described. They include a simple extraction in 0.85% NaCl (Bozicevich, 1938), a fairly involved extraction of an acid soluble fraction (Melcher, 1943), and collection of antigenic larval metabolic products (Sadun and Norman, 1957). These and others were reviewed in 1960 by Sadun.

Detection of metabolic antigens by the use of agar gel double diffusion method has been described (Olson et al., 1959). Their micro agar gel technique detected immunoprecipitins when hyperimmune rabbit serum was diffused against a micro culture of living larvae, a sonicated larval preparation and a preparation of excretion and secretion products. Some of the bands were formed in as few as 17 hours.

Gel diffusion as a procedure for analyzing and standardizing of Trichinella extracts has also been described (Wodehouse, 1956).

MATERIALS AND METHODS

Source and Maintenance of Infection

The strain of T. spiralis used in this work was obtained from W. J. Zimmermann (Iowa State University) and maintained in white rats and mice from the rodent colony of the Department of Infectious Diseases. Larvae for inoculating stock animals were obtained by digesting ground tissue in a mixture of 1% pepsin - 1% HCl. Twenty ml of fluid were used for each gram of ground tissue. The mixture was incubated at 37 C for 4½ hours with occasional mixing and then poured into graduated cylinders and allowed to settle at room temperature for 2-3 hours. The sedimented larvae were then collected, washed to remove debris, and suspended in a graduated centrifuge tube. Two-tenths ml of the larval suspension was placed on a glass microscope slide and the larvae counted. The average of four such counts was used to determine the total number of larvae present. A suspension containing the desired number of larvae was introduced into the stomach by means of a miniature Whitlock nozzle attached to a glass syringe (Leland, 1957). Doses for stock rats ranged from 750 to 7,500 larvae.

Sex Determination of Larvae

At very low dose levels it is important that both male and female larvae be included in the infecting dose. Therefore, a trial was set up to test the sexing technique described by Villella (1966). Larvae were prepared as for stock animals. Seventy-five female larvae were selected and placed in a labeled centrifuge tube. Two other tubes were prepared, each contained 75 randomly selected unsexed larvae. Each of these larval

suspensions was used to inoculate a young male white mouse. Thirty-seven days later the mice were killed and the evicerated carcasses ground and digested as previously described. The larvae were collected and counted.

Experimentally Infected Pigs

The 5 pigs used for this experiment were 10 weeks of age when purchased. They were littermate Chester Whites, ranging in weight at the time of exposure from 19 to 33 pounds. All were vaccinated against hog cholera and all were negative on stool examination for internal parasites at the start of the experimental period.

The pigs were housed indoors in concrete floored, individual pens during the entire experimental period. The ration consisted of commercial hog pellets. Water was available ad libitum.

The larvae used to infect the pigs were derived from a stock rat infected 30 days earlier. The doses given were as follows:

Pig #	Larvae
1	0
2	10 (6 female larvae, 4 male larvae)*
3	50
4	100
5	500

The larvae used to infect pigs #2, 3, and 4 were counted directly while the dilution counting method was used to prepare the dose for pig #5. Only the

*Sexed by technique of Villella (1966).

larvae used to inoculate pig #2 were selected as to sex. Random selection of larvae for other inoculations was depended upon to provide the proper sex ratio.

The larvae were placed in centrifuge tubes containing distilled water, centrifuged (750 X G/5 min.) and all but 2 ml of the water removed using a Pasteur pipette. The dose was administered using a miniature Whitlock nozzle. The apparatus was rinsed 3 times and the rinsings also deposited in the stomach to insure quantitative delivery.

Infectivity Control

To demonstrate infectivity of the larvae used to inoculate the test pigs, mice were also inoculated with a portion of the larval suspension. Dose levels and preparation methods were identical. Dosing procedure was the same except that a smaller miniature Whitlock nozzle fitted with a tuberculin syringe was used. The volume of fluid used was also reduced.

After a minimum of 30 days these mice were sacrificed to determine if infection had occurred. Diaphragm samples were examined under the disection microscope by compressing the tissue between two microscope slides. In addition, 4 mice, one representing each dose level, were evicerated, skinned, ground, and digested as described earlier. An estimate of the number of larvae in each of these 4 mice was made by counting aliquots of a known dilution.

Tissue Examination of Experimentally Infected Pigs

The experimental pigs were slaughtered between 11 and 13 weeks after exposure. Pig #1 was electrically stunned then exsanguinated. Pig #2 was

given 20 cc of Barb-Euthol* intratesticularly. As soon as anesthesia was evident the pig was exsanguinated. Pig #3 (a female) was given 20 ml of Barb-Euthol IV.* Before the completion of the injection, the animal was clinically dead. The jugular veins were severed but only a small volume of blood was obtained.

Pig #4 was slaughtered using the same method described for pig #2. Pig #5 (a female) was stunned by a blow to the head and then exsanguinated. The diaphragm was taken for examination by digestion. Two examinations by digestion were conducted on each sample. The method used was essentially that described by Zimmermann (1967) in which he recommends the following:

1. Samples of the pillars of the diaphragm weighing from 5-6 grams were taken from each of the pigs in the lot (20 pigs).
2. The pooled sample was ground using a mechanical food chopper.
3. The ground sample (approximately 100 gm) was placed in a beaker containing about 3 liters of 1% pepsin - 1% hydrochloric acid digestion fluid. The fluid was pre-warmed to 37 C.
4. The sample was continuously agitated and kept at 37 C for 12 hours. At the end of the digestion period the mixture was allowed to settle at 37 C for 1 hour, then about 2/3 of the supernatant was siphoned off.
5. The remaining fluid was poured through an 80 mesh sieve into a 10 inch Baermann funnel. The beaker was rinsed and rinsings were added to the funnel. The fluid was allowed to settle at 37 C for 1 hour.

---

*Barb-Euthol, Haver-Lockhart, Kansas City, Mo.

6. The clamp was opened and a 5 inch Baermann funnel was filled. This fluid was allowed to settle for 1 hour at 37 C.
7. A portion of the fluid was drawn off into a ruled Syracuse watch glass or ruled petri dish for microscopic examination. Examinations were made at 27X or 80X magnification.
8. If any larvae were found, the lot (20 swine) was positive and individual samples of 40-50 gm were run.

Zimmermann's procedure was modified as follows:

1. Fifty gram samples representing only one animal were used in lieu of 20 - 5 gram samples.
2. Two digestion periods were used. Group A samples were allowed to digest for 12 hours. Group B samples were allowed to digest until no large pieces of lean tissue were visible (approximately 3 hours).
3. Screens were not used.
4. Eight inch funnels were used in place of ten inch and five inch funnels.
5. All of the fluid in the second funnel (approximately 1,500 ml) was examined and the number of larvae recovered in each 100 ml aliquot was recorded. The numbers were totaled and larvae/gram yields calculated.

Charcoal Card Agglutination Testing

The Charcoal Card Test* (Anderson et al., 1963) was used to test serum

*Reagents and equipment for this test were provided by Hynson, Westcott and Dunning, Baltimore, Maryland.

samples from the experimental pigs, slaughter pigs, and experimentally infected rats. Blood samples were taken weekly for 10 weeks from the experimental pigs by venipuncture beginning on the day of exposure. Known positive and negative rat sera were run with each group of samples from the experimental animals and served as controls.

Tests were also conducted on 52 serum samples taken from pigs butchered at the University's slaughter house. Blood was obtained either by venipuncture or was taken at the time of exsanguination.

One hundred forty-two additional serum samples were obtained by collecting heart blood from swine slaughtered at a cooperating commercial meat plant.*

Positive and negative control serum was derived from blood taken from rats using a cardiac puncture technique. Rats were anesthetized with sodium pentabarbital and chloroform before bleeding. Tissue samples from these rats were examined to insure that they were truly positive or negative.

All blood samples were allowed to clot at room temperature for from 1 to 12 hours then placed in the refrigerator for at least 1 hour. The resulting serum was then tested as follows:

1. A drop (0.03 ml) of serum was placed in the ring on the trichinosis test card using a capillary pipette.
2. One drop of antigen was placed in the test ring. The special dispenser delivers 1/66 ml.
3. Serum and antigen were mixed and spread over the entire ring using a flat stirrer.

---

*Mauer Neuer Meat Company, Arkansas City, Kansas.

4. The card was rotated in a 3/4 inch circle for 8 minutes on a mechanical rotator at 100 R.P.M.
5. Tests were read wet and classed as reactive (+) or non-reactive (-).

Test results were recorded and the test cards dried and stored for reference. Portions of the above sera were stored frozen at -20 C for later use in gel diffusion testing.

A 5 gm portion of the pillar of the diaphragm was examined from all pigs reacting positively to the serum test to either confirm or refute the serological findings. Digestion procedures were essentially the same as those used on the test pig diaphragms except that the settling periods and funnels were eliminated because the volume of fluid to be examined was so small (approximately 200 ml).

Gel Diffusion Antigen Preparation

Sonicated larval antigen was prepared as follows:

1. Approximately 200,000 larvae in 20 ml of 0.85% NaCl were disrupted by sonic waves generated by a 100 watt 20 K.C. electrorestrictive sonic generator.* Exposure time was 8 minutes.
2. The saline suspension was extracted for 24 hours at 4 C.
3. Particulate matter was removed by centrifugation (1500 X G for 30 minutes at 4 C).
4. Supernatant solution (sonicated antigen solution) was stored at -20 C until used.

*Sonifer model W-104C, Branson Sonic Power Co.

Excretion and secretion antigen was prepared by placing approximately 5,000 larvae in 2 ml of nutrient medium. The formula for this medium is given below.

50 ml	CEE_{50}
15 ml	swine serum
15 ml	sodium caseinate
5 ml	vitamin mixture
5 ml	liver extract
1 ml	antibiotic mixture
9 ml	BSSA
100 ml	Total

A detailed description of the components of the medium and its preparation has been published (Leland, 1963).

The larvae were incubated at 37 C in this media for 3 days. Only cultures with 50% or more larvae alive after 3 days of cultivation were used. Useable cultures were centrifuged at 1500 X G for 30 minutes at 4 C. The supernatant was tested for pH and sterility. This supernatant, which presumably contained excretion and secretion products, was then stored at -20 C in a screw cap vial.

Culture of Trichinella spiralis

Two other modified media were used in cultivation attempts. The formula given earlier was modified by substituting 3.1 ml of media 199* (Morgan

*Produced by Biological Associates, Baltimore, Maryland.

et al., 1950) and 5.9 ml sterile distilled water for the 9 ml of BSSA. Modifying the basic medium by adding glucose at the rate of 10 mg/ml was also tried.

Larvae for use in culturing were freed from the host tissue by the same digestion procedure used to prepare larvae for inoculation of stock animals except that sterile water was used for rinsing. The larvae were washed 6X in sterile water then counted and approximately 2,000 placed in a screw top tube (16mm X 125mm) containing 2 ml of medium. Tubes were incubated at 38.5 C in a roller tube apparatus (12 revolutions per hour).

Gel Diffusion Testing

The gel used for the double diffusion test was made up of 2.0% Noble Agar* in 0.85% saline. The mixture was autoclaved at 15 lb psi for 30 minutes to insure uniformity and sterility.

Ten ml of the hot gel was placed in a 60mm X 15mm disposable plastic petri dish. The gel was allowed to set overnight before wells were cut using a number 4 cork borer (10mm in diameter). The interwell distance was 5mm. Six wells were symetrically placed around a center well. The peripheral wells contained serum samples to be tested and either sonicated larval antigen or excretion and secretion antigen was placed in the center well.

The plates were placed in a plastic box with a lid to minimize evaporation. The plates were kept at room temperature for 72 hours.

*Special Agar-Noble, Difco Laboratories, Detroit, Michigan.

RESULTS

Sex Determination Criteria

The sexing trial demonstrated that sorting of larvae using the criteria proposed by Villella (1966) was at least partially effective. A total of approximately 3,000 larvae were recovered from the mouse which received 75 selected female larvae while approximately 7,000 and 7,500 larvae respectively were recovered from the two mice which had been given 75 randomly selected unsexed larvae.

Infectivity Control

Infectivity was unimpaired by the preparation and inoculating procedures as evidenced by the results shown in Figure 1.

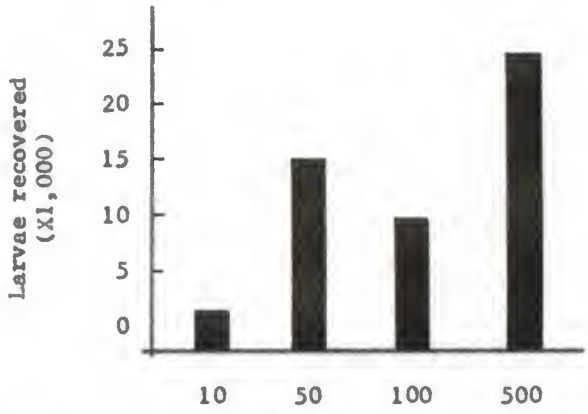

Figure 1.--Larvae recovered from Mice Used to Demonstrate that Infectivity of Inoculum was Unimpaired by Preparation and Inoculation Procedures.

The information given in Figure 1 is based on one set of 4 mice. The other set of 4 mice were checked by examining the diaphragm muscle using the

steroscopic disecting microscope. All of the samples checked contained larvae.

Tissue Examination of Experimentally Infected Pigs

Diaphragm samples from the test pigs were digested using the two periods of digestion. The results are shown in Table 2.

INFECTIVE LARVAE INOCULATED	LARVAE RECOVERED/GRAM OF DIAPHRAGM		
	GROUP A (DIGESTION FOR 12 HOURS)	GROUP B (DIGESTION TO VISUAL END POINT)	AVERAGE
0	0.00	0.00	0.00
10	0.52	0.60	0.56
50	0.00	0.02	0.01
100	5.77	5.45	5.61
500	87.34	81.48	84.41

TABLE 2.--Larvae recovered/gram of Diaphragm from Swine Experimentally Infected with Trichinella spiralis.

In addition to the total larvae recovered, a record was kept of the number of larvae recovered from 100 ml fractions. These observations are summarized in Table 3.

INFECTIVE LARVAE INOCULATED	SAMPLE GROUP	PERCENT OF TOTAL LARVAE RECOVERED IN: FIRST 100 ml	LAST 100 ml
10	A	88	4
	B	77	10
50	A	--	--
	B	100*	--
100	A	35	42
	B	60	12
500	A	78	4
	B	69	25

TABLE 3.--Effect of Varied Digestion Period on the Percentage of Larvae Recovered in the First and Last 100 ml Fractions of the Tissue Digest.

A = Digestion 12 hrs.

B = Digestion to visual end point.

*One larva recovered.

Charcoal Card Agglutination Testing

At no time during the 12 weeks of testing did any of the serum samples from the experimental swine react positively with the charcoal card agglutination test.

Fifty-two slaughter pigs from the University abattoir were checked for trichinosis using the Charcoal Card Test. None of the sera tested gave a positive reaction. Sera from 142 slaughter pigs obtained from the commercial plant were tested. Two of these samples reacted positively to the test. Diaphragm samples from these two CCT positive animals plus 24 CCT negative animals were examined by the digestion procedure. No larvae were

found in any of the samples.

Culture of Trichinella spiralis

The formula which gave the best results of the three media tried was given earlier. The modified Ae medium (Leland, 1963) supported life for a maximum of 12 days. Shrinkage of some of the larvae while in culture was observed. Single sheaths were formed by most larvae but occasionally double or multiple sheaths were seen. An occasional larvae would develop enlargements in the tail region which suggested the beginning of sexual differentiation. The cones typical of adult male Trichinella failed to develop and adult females were not produced.

Gel Diffusion Testing

Serum samples from pig #5 (dose 500), pig #1 (control), and a heavily infected rat were examined using the gel diffusion technique described earlier. Pre-exposure, 4 week, and 8 week serum samples from pig #1 and #5 were tested. The excretion and secretion, and sonicated larval antigens described in the materials and methods section were used in performing this test. No lines of precipitate were produced with pig sera but precipitate was produced with the rat serum. Two bands were produced by the excretion and secretion antigen while only one was produced by the sonicated larval antigen.

DISCUSSION

This study was designed to examine the larval inoculation and recovery relationships of T. spiralis in swine at low inoculation levels, and the ability of the Charcoal Card Test to detect these light infections in swine. In addition, in vitro culture of T. spiralis was undertaken in an effort to produce antigens which could be used in a gel diffusion system. Sensitivity of a gel diffusion technique as a method of detecting swine with naturally occurring levels of Trichinella infection was investigated.

When attempting to produce infection with very low numbers of larvae the sex ratio of the larvae in the dose is of paramount importance. Even though the results of Villella (1966) were not duplicated in this trial, the effect of sorting was marked. The yield of the mice which received the selected female larvae produced only 41% as many muscle larvae as did the mice which received the 75 randomly selected unsexed larvae. The sex ratio of 2 females : 1 male was selected because it was the value generally reported in the literature (Roth, 1938).

The infectivity control data indicated infective larvae were present in all of the doses. There was a lower than expected yield in the mouse which received the dose of 100 larvae. This relatively lower yield could be due to any one of many factors some of which are listed below:

1. Damage to the larvae by preparation, handling, or dosing procedures.
2. An unfavorable sex ratio.
3. Variation in the susceptibility of the individual mouse.

The tissue examinations were undertaken to determine the inoculation-recovery relationship in the experimental pigs. Two digestions were made using different time periods to see if the yield was affected. This

variable was not shown to affect the yield either one way or another, therefore an average yield was calculated. The percentage of variation was very small. With inoculation of 10 larvae the variation in recovery was ± 7.1%. With inoculation of 100 larvae the variation in recovery was ± 2.8%. With inoculation of 500 larvae the variation in recovery was ± 0.1%.

The inoculation-recovery data is in general agreement with figures taken from the literature as shown in Table 4.

NUMBER OF PIGS STUDIED	LARVAE INOCULATED	RECOVERY LARVAE/GRAM	LITERATURE SOURCE
1	10	0.56	Present Study
1	50	3.8	Hill, 1957
1	100	5.6	Present Study
12	100	13	Suessenguth, 1965
1	500	84	Present Study
3	500	31	Scholtens, 1966
19	500	36.7	Suessenguth, 1965
3	1,000	61	Scholtens, 1966
22	1,000	93.2	Suessenguth, 1965
10	50,000	1,444	Olsen, 1964
3	100,000	3,766	Scholtens, 1966
1	100,000	910	Olsen, 1964
1	150,000	3,503	Olsen, 1964

TABLE 4.--Trichinella Inoculation-Recovery Data from Various Investigators.

The dose of 50 larvae (Table 2) did not produce an infection compatible with other levels of inoculation. Several of the possible reasons for this were given earlier but one other possibility must be considered.

The chemicals used to euthanize pig #3 (Table 2) may also have been lethal to the larvae in the tissue. The dead larvae could have been subsequently digested in the recovery procedure. Recall that this pig (given Barb-Euthol IV) died so rapidly that only a small amount of blood was obtained when exsanguination was attempted.

The effect of varied digestion time on the percent of larvae in certain fractions of the digestion fluid is shown on Table 3. There was no consistant difference between the two digestion periods. One important fact is apparent, namely, that if a sample contains larvae, there will be some larvae in the first 100 ml fraction of the digestive mixture. Therefore if samples are digested using the technique of Zimmermann (1967) and no larvae are found in the first 100 ml, the results of this study indicate that the remainder of the sample will also be negative. This observation is in agreement with the report by Zimmermann (1967).

The failure of the Charcoal Card Test to detect antibody in serum from pig #5 (dose 500) during the test period was not in agreement with the observations of Scholtens et al., (1966) who reported positive CCT tests in three pigs 28, 43, 64, and 83 days after being inoculated with 500 larvae.

If the reported incidence, 0.5% $\pm$ 0.26% (Jefferies et al., 1966) applied directly to the slaughter pigs tested, the number of infected swine should range from 0 to 2. Therefore finding 192 negatives and 2 positives using the CCT might have been very encouraging had the tissues from the positive pigs contained larvae. They did not and no adequate explanation for the 2 apparently false positive tests can be made. The possibility of these reactions being due to non-specificity of the test must be seriously considered.

Berntzen (1965) has described a successful though very complex system which reportedly will support Trichinella spiralis from excysted larvae to sexually mature adults. The roller tube system, used in this work because of its relative simplicity, deserves additional study as a possible method for culturing T. spiralis even though no comparable results were achieved in this study. The larvae placed into culture did, however produce secretions and/or excretions as evidenced by the change of the buffered media from pH 7.25 to pH 6.83. Bacterial contamination was not detected in these cultures. The formation of precipitin lines when serum from the heavily infected rat was diffused against the two antigen preparations is interpreted as evidence that the two preparations did, in fact, contain precipitatable antigens.

CONCLUSIONS

This study has added to the body of knowledge relating to the inoculation-recovery relationship of Trichinella in swine at low inoculation levels. The Charcoal Card Test was not found sensitive enough to detect swine with infections resembling those most commonly occurring in the United States today. False positive reactions to the CCT were found. It is therefore concluded that until such time as a more reliable test becomes available, tissue digestion techniques continue to be the best method of detecting naturally occurring trichinosis in swine.

Additional work on serological diagnosis is indicated and a method of detecting trichinosis in live swine is as much of a challenge now as it has been in the past. The need for a less complex system for the successful cultivation of T. spiralis continues to exist and the development of such a system is a worthy goal.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Doctor S. E. Leland, Jr., Professor of Parasitology in the Department of Infectious Diseases for his advice and encouragement throughout the course of this study.

Special acknowledgment of the advice, assistance and active interest in this study is due Doctor D. C. Kelley, Associate Professor in the Department of Infectious Diseases and to Doctor Don Kropf, Associate Professor, Department of Animal Husbandry.

Gratitude is extended to all those other people in the Department of Infectious Diseases and the Department of Pathology who have offered encouragement and assistance.

Special gratitude is extended to the Mauer Nauer Meat Company of Arkansas City, Kansas for their help in obtaining blood and diaphragm samples from slaughter swine used in this study.

Special gratitude is also extended to the Warner and Hynson, Westcott, and Dunning Inc. of Baltimore, Maryland who supplied the Charcoal Card Test materials, the card test rotating machine, and some other reagents. These materials were supplied free of charge and this support was greatly appreciated.

Dr. W. J. Zimmermann is extended special gratitude for supplying the initial stock of T. spiralis larvae used in this study.

The author is indebted to the Office of the Surgeon General, Department of the Army, for providing the opportunity to participate in this program of study at Kansas State University.

LITERATURE CITED

Anderson, R. J., Sadun, E. H., and Schoenbelcher, M. J.: Cholesterol-Lecithin Slide and Charcoal Card Flocculation Tests Using an Acid Soluble Fraction of Trichinella spiralis. Journal of Parasitology, 49, (1963): 642-647.

Annon.: Laboratory Diagnosis of Parasitic Diseases. U.S. Department of Health, Education and Welfare, Public Health Service, National Communicable Disease Center, Atlanta Georgia, (1967).

Annon.: Trichinosis Surveillance. U.S. Department of Health, Education and Welfare, Public Health Service, National Communicable Disease Center, Atlanta, Georgia, (1968).

Bachman, G. W.: A Precipitin Test in Experimental Trichinosis. Journal of Preventive Medicine, 3, (1929): 465-469.

Berntzen, A. K.: In Vitro Cultivation of Trichinella spiralis. Journal of Parasitology, 48, Suppl., (1962): 48.

Berntzen, A. K.: Comparative Growth and Development of Trichinella spiralis In Vitro and In Vivo with a Redescription of the Life Cycle. Experimental Parasitology, 16, (1965): 74-106.

Bozicevich, J.: Studies on Trichinosis, XII. The Preparation and Use of an Improved Trichina Antigen. Public Health Reports, 53, (1938): 21-30.

Bozicevich, J., Tobic, J. E., Thomas, E. H., Hoyem, H. M., and Ward, S. B.: Rapid Flocculation Test for the Diagnosis of Trichinosis. U.S. Public Health Service Report, 66, (1951): 806-814.

Campbell, C. H.: The Antigenic Role of the Excretions and Secretions of Trichinella spiralis in the Production of Immunity in Mice. Journal of Parasitology, 41, (1955): 483-491.

Chandler, H. C., and Read, C. P.: Introduction to Parasitology 10th Ed. John Wiley and Sons Inc., New York, New York.

Cram, E. B.: Trichinella spiralis: An introduction to Nematology. M. B. Chitwood, Babylon, New York (1941), from Gould (1945).

Denham, D. A.: Infections with Trichinella spiralis Passing from Mother to Filial Mice Pre and Post Natally. Journal of Helminthology, 41, (1966): 291-296.

Friedreich, N.: Ein Beitrag zur Pathologie der Trichenkrankheit beim Menschen. Virchows Arch. F. Path. Anat., 25, (1862): 399-413, from Gould (1945).

Gordon, J. E.: Control of Communicable Diseases in Man. American Public Health Association, New York (1965).

Gould, S. E.: Trichinosis. Charles C. Thomas, Springfield, Illinois (1945).

Herbst, G.: Beobachtungen Uber Trichina spiralis in Betreff der Uebertragung der Eigeweidewurmer. Nachrichten V. D. Georg. August Univ D. D. Konigl Gesellschaft D. Wissenschaften, Gottingen (1851): 260-264, from Gould (1945).

Hill, C. H.: The Recovery of Encapsulated Larvae of T. spiralis Relatively Free of Muscle Tissue. Proceedings of the Helmenthological Society of Washington, 18, (1951): 114-120.

Hill, C. W.: Distribution of Trichinella spiralis in the Organs of Experimentally Infected Swine. Journal of Parasitology, 43, (1957): 574-577.

Innella, F., and Redner, W. J.: Latex Agglutination Serologic Test for Trichinosis. J.Am.M.A., 171, (1959): 885-887.

Jefferies, T. C., Beal, V., Murtishaw, T. R., and Zimmermann, W. J.: Trichinae in Garbage Fed Swine. Proc. U.S. Livestock San. A. (1966): 349-357.

Kagen, I. G.: Trichinosis: A Review of Biologic, Serologic, and Immunologic Aspects. Journal of Infectious Diseases, 107, (1960): 65-93.

Kagen, I. G., and Bargai, U.: Studies on the Serology of Trichinosis with Hemagglutination, Agar Diffusion Tests and Precipitin Ring Tests. Journal of Parasitology, 42, (1956): 237-245.

Kim, C. W.: The cultivation of T. spiralis In Vitro. American Journal of Tropical Medicine and Hygiene, 10, (1961): 742-747.

Kim, C. W.: Further Study on the In Vitro Cultivation of Trichinella spiralis. American Journal of Tropical Medicine and Hygiene, 11, (1962): 491-496.

Kozar, Z., and Kozar, Z.: A Comparison of Infectivity and Pathogenicity of T. spiralis strains from Poland and Kenya. Journal of Helmenthology, 39, (1965): 19-34.

Lamb, G. A., Kagen, I. G., Scholtons, R., Preizler, J.: Evaluation of Intradermal and Serological Tests in a Large Outbreak of Trichinosis. American Journal of Hygiene, 80, (1964): 235-241.

Leidy, J.: Remarks on Trichina. Proceedings of the Academy of Anatomical Sciences of Philadelphia, 3, (1846): 107-108 from Gould (1945).

Leland, S. E., Jr., and Drudge, J. H.: Studies on Trichostrongylus axei (Cobbold, 1879). II. Some Quantitative Aspects of Experimental Infection in Rabbits. Journal of Parasitology, 43, (1957): 160-166.

Leland, S. E., Jr.: Studies on the In Vitro Growth of Parasitic Nematodes I. Complete or Partial Parasitic Development of Some Gastrointestinal Nematodes of Sheep and Cattle. Journal of Parasitology, 49, (1963): 600-611.

Levine, A. J.: Recovery of Trichinella spiralis Larvae in Early Stages of Infection. Journal of Parasitology, 27, (1941): 107-113.

McCoy, O. R.: The Development of Trichinae in Abnormal Environments. Journal of Parasitology, 22, (1936): 54-59.

McCoy, O. R.: Rapid Loss of Trichinella Larvae Fed to Immune Rats and Its Bearing on the Mechanism of Immunity. American Journal of Hygiene, 32, (1940): 103-116.

Melcher, L. R.: An Antigenic Analysis of Trichinella spiralis. Journal of Infectious Diseases, 73, (1943): 31-39.

Mills, C. K., and Kent, N. H.: Excretions and Secretions of Trichinella spiralis and Their Role in Immunity. Experimental Parasitology, 16, (1965): 300-310.

Morgan, J. F., Morton, H. J., and Parker, R. C.: Nutrition of Animal Cells in Tissue Culture. I. Initial Studies on a Synthetic Medium. Proc. Soc. Exptl. Biol. & Med., 73, (1950): 1-8.

Most, H.: Trichinellosis in the U. S.--Changing Epidemiology During the Past 24 Years. J.Am.M.A., 193, (1965): 871-873.

Norman, L., and Kagen, I. G.: Bentonite, Latex and Cholesterol Flocculation Tests for the Diagnosis of Trichinosis. Public Health Report, 78, (1963): 227-232.

Oliver-Gonzales, J.: The In Vitro Action of Immune Serum on the Larvae and Adults of Trichinella spiralis. Journal of Infectious Diseases, 67, (1940): 292-300.

Olsen, B. S., Villella, J. B., and Gould, S. E.: Distribution of Trichinella spiralis in Muscles of Experimentally Infected Swine. Journal of Parasitology, 50, (1964): 489-495.

Olson, L. J., Richards, B., and Ewert, A.: Detection of Metabolic Antigens from Nematode Larvae by a Microculture-Agar Gel Technique. Texas Reports on Biology and Medicine, 18, (1959): 254-259.

Podhajecky, K.: Contributions to the Epizootology of Trichinellosis. Proceedings of the First International Congress of Parasitology, II, (1964): 665.

Portnoy, J., Brewer, J. H., and Brewer, H. A.: Rapid Plasma Reagin Card Test for Syphilis and Other Treponematoses. Public Health Reports, 77, (1962): 645-652.

Roth, H.: Experimental Studies on the Course of Trichina Infection in Guinea Pigs. I. The Minimum Dose of Trichina Larvae Required to Produce Infection of the Muscles; with an Account of the Potential Productiveness of the Female Trichina. American Journal of Hygiene, 28, (1938): 85-103.

Sadun, E. H., and Allain, D. S.: A Rapid Slide Hemagglutination Test for the Detection of Antibodies to Trichinella spiralis. Journal of Parasitology, 43, (1957): 383.

Sadun, E. H., and Norman, L.: Metabolic and Somantic Antigens in the Determination of the Response of Rabbits to Graded Infections with Trichinella spiralis. Journal of Parasitology, 43, (1957): 236-245.

Sadun, E. H.: Recent Advances on the Serological Diagnosis of Trichinosis. Proceedings of First International Conference on Trichinosis at Warsaw, Poland, (1960).

Sadun, E. H., Anderson, R. J., and Williams, J. S.: Florescent Antibody Test for the Serological Diagnosis of Trichinosis. Experimental Parasitology, 12, (1962): 423-433.

Scholtens, R. G., Katen, I. G., Quist, K. D., and Norman, L. G.: An Evaluation of Tests for the Diagnosis of Trichinosis in Swine and Associated Quantitative Epidemiological Observations. American Journal of Epidemiology, 83, (1966): 489-500.

Schwartz, B., and McIntosh, A.: Skin Reactions in Experimental Trichinosis in Pigs. Journal of Parasitology, 16, (1929): 104-105, from Gould (1945).

Spindler, L. A., Cross, S. X., and Avery, J. L.: Results of Intracutaneous Tests for the Detection of Trichina Infections in Swine. Proc. Helminthol. Soc. Washington, 8, (1941): 1-5, from Gould (1945).

Spindler, L. A.: Transmission of Trichinae to Swine through Feces. Journal of Parasitology, 39, Suppl., (1953): 34.

Strafuss, A. C., and Zimmermann, W. J.: Hematological Changes and Clinical Signs of Trichinosis in Swine. Am. J. Vet. Res., 28, (1967): 833-838.

Strobel, H.: Die Serodiagnostik der Trichinosis Munch. Med. Wchnschr., 58, (1911): 672-674, from Gould (1945).

Suessenguth, H., and Kline, G. S.: A Simple Rapid Flocculation Slide Test for Trichinosis in Man and Swine. American Journal of Clinical Pathology, 14, (1944): 471-484.

Suessenguth, H., Schnurrenberger, P. R., Bauer, A., Wentworth, F. W., and Masterson, R. A.: Swine Trichinosis. Serological Reactivity in Experimental Infection with Small Numbers of Larvae. Am. J. Vet. Res., 26, (1965): 1298-1302.

Sulzer, A. J., and Chisholm, E. S.: Comparison of I.F.A. and Other Tests for T. spiralis Antibodies. Public Health Report, 81, (1966): 729-734.

Thornbury, F. J.: The Pathology of Trichinosis. Original Observations. Univ. Med. Mag., 10, (1897): 64-79, from Gould (1945).

Villella, J. B.: Morphological Criteria for Distinguishing the Sex of Trichinella spiralis Larvae from Muscle. Journal of Parasitology, 52, (1966): 908-910.

Weller, T. H.: The Development of T. spiralis in Roller Tube Tissue Culture. American Journal of Pathology, 19, (1943): 503-516.

Wodehouse, R. P.: Antigenic Analysis and Standardization of Trichinella Extract by Gel Diffusion. Annals of Allergy, 14, (1956): 121-138.

Zenker, F. A.: Ueber die Trichinen Krankheit des Menschen. Virchows Arch. F. Path. Anat., 18, (1860): 561-572, from Gould (1945).

Zimmermann, W. J., and Hubbard, E. D.: Wildlife Reservoirs of Trichinella spiralis. Proceedings of the American Veterinary Medical Association, (1963): 194-199.

Zimmermann, W. J., and Brandly, P. J.: Current Status of Trichinosis in U.S. Swine. Public Health Report, 80, (1965): 1061-1066.

Zimmermann, W. J.: Can We Eradicate Trichinosis? J.A.V.M.A., 149, (1966): 319-320.

Zimmermann, W. J.: A Pooled Sample Method for Post-Slaughter Detection of Trichiniasis in Swine. Proc. U.S. Livestock San. A., (1967): 358-366.

AN EVALUATION OF SOME METHODS FOR DETECTING LOW LEVELS OF INFECTION AND INOCULATION-RECOVERY RELATIONSHIPS OF *TRICHINELLA SPIRALIS* IN SWINE

by

MAX ELMER COATS JR.

D. V. M., Colorado State University, 1963

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Pathology

Department of Infectious Diseases

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1969

This study evaluates some methods of detecting low levels of infection with Trichinella spiralis in swine. In vitro cultivation of T. spiralis was attempted and the excretions and secretions produced were used in a gel diffusion system. Sensitivity of a gel diffusion technique as a method of detecting low levels of T. spiralis infection in swine was investigated.

This study adds to the knowledge of inoculation/recovery relationship of T. spiralis infection in swine. The results of the serological testing suggest that the Charcoal Card Test is not sensitive enough to detect the low levels of infection in swine which represent the most commonly occurring form in the United States today. False positive reactions to the Charcoal Card Test were also shown to occur. A tissue digestion procedure was investigated and appears to still be the best method of detecting naturally occurring trichinosis in swine. The in vitro cultivation and gel diffusion procedures were not sufficiently perfected to detect the low levels of infection considered in this study.